STUDIES ON SPERMICIDAL ACTIVITY OF SURFACTANTS. I. CORRELATION BETWEEN SPERMICIDAL EFFECT AND PHYSICO-CHEMICAL PROPERTIES OF *p*-MENTHANYLPHENYL POLYOXYETHYLENE (8.8) ETHER AND RELATED SURFACTANTS

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The spermicidal activities of seven nonionic surfactants against human spermatozoa were objectively determined by a statistical method, and two types of ionic surfactants were also studied for purposes of comparison. The nonionic surfactants, *p*-menthanylphenyl polyoxyethylene adducts used in this work were synthesized from turpentine oil. These surfactants were used in the experiment after a careful confirmation of their chemical composition and molecular weight distribution. The spermicidal activities of the surfactants in terms of minimum concentrations to accomplish irreversible immobilization of all sperms in zero time was, in decreasing order, nonionic, cationic and anionic. When the most commonly used nonionics alone were considered, the sequence of decreasing spermicidal potency was isononylphenyl polyoxyethylene (9.0) ether (nonoxynol-9), *p*-menthanylphenyl polyoxyethylene (8.8) ether (menfegol), isooctylphenyl polyoxyethylene (9.0) ether (octoxynol-9).

The surface tension and wetting time of these surfactants were determined with a view to finding physico-chemical measures of their spermicidal activity. In the statistical analysis regarding all the nine surfactants, there was a significant correlation ($p < 0.05$) between spermicidal potency and critical micelle concentration (cmc) whereas there was no significant relationship between spermicidal potency and cmc regarding the seven nonionics. There was again no significant correlation between spermicidal potency and wetting time in all but nonionics with a common hydrophobic structure. As neither surface tension nor wetting time appeared to be a valid factor, general measure of spermicidal potency of nonionic surfactants, the partition ratios between *n*-octanol or *n*-hexane and water was determined. As a result, it was revealed that there was a significant correlation ($p < 0.05$) between spermicidal potency and partition coefficient, when four types of nonionic surfactants with different hydrophobic group, which were chosen from among the seven nonionics, were tested with *n*-hexane.

The close relationship between spermicidal activity and partition coefficient suggests that the spermicidal activities of these surfactants are associated with their structural affinity to the lipids of spermatozoal cell membrane.

Keywords — human spermatozoa; spermicide; vaginal contraceptive; spermicidal effect; surface tension; wetting time; menfegol; nonoxynol-9; octoxynol-9

INTRODUCTION

Since early 1930, observations of the inhibitory effect of surfactants on the motility of spermatozoa from mammals,¹⁾ such as guinea-pig,

rat, ram and bull, and of human spermatozoa²⁾ have been reported. Nevertheless, the mechanism of spermicidal activity of surfactants has not been fully elucidated.

Although it was suggested earlier that the substances which could lower surface tension ought to be good spermicides, as for the surface tension of spermicides, there had been only a few published studies by Baker *et al.*,^{2a)} Swayne *et al.*^{2b)} and by Harvey and Stuckey.^{2c)} In the studies of those investigators, it is stated that they had a difficulty in definitely demonstrating the correlation between the spermicidal activity and surface tension.

However, considering the possibility that the inhibition of spermatozoal motility caused by the surface-active spermicides in the primary stage of their action might be due to their physico-chemical properties, the work of those investigators was experimentally inconclusive. For example, the chemical compositions of their test samples were not sufficiently clear and the use of electrolytes, including a physiological saline solution, as diluents for the samples and semen could have inadvertently altered the physico-chemical properties of the ionic surfactants. It would appear that the spermicidal activity of the surfactants could not be considered apart from their important functional property, the lowering of surface tension, and that a further investigation was required to clarify the matter.

In the previous paper,³⁾ the authors described that the newly synthesized nonionic surfactant, *p*-menthanylphenyl polyoxyethylene (9.0) ether with the characteristic hydrophobes derived from turpentine oil, was satisfactory in the efficacy and safety aspects as a spermicide. Subsequently, they obtained an indication that, the spermicidal activity of the various ethylene oxide (EO) adducts of *p*-menthanylphenol might be related to the wetting efficiency, which is an inherent physical property of surfactants.^{4,5)}

Hence, in the present work, the chemical compositions of all samples used were first clarified as far as possible and non-electrolytes such as isotonic glucose solution were used as diluents for the test materials. Furthermore, an objective method was adopted to evaluate the spermicidal

effect and the experiments were carefully designed to assess the effects of physico-chemical properties including surface tension and partition ratios between water and organic solvents.

In addition, the work on the effect of the rate of wetting on spermicidal activity of *p*-menthanylphenyl polyoxyethylene adducts reported in previous paper was extended to other surface-active spermicides to confirm the applicability of the relationship.

MATERIALS AND METHODS

Chemicals — Using the previously described method,^{6,7)} 6.0- to 20.0-mol EO adducts to *p*-menthanylphenol (TS-60, TS-71, TS-80, TS-112, TS-120, TS-131, TS-140, TS-150, TS-165 and TS-200) and *p*-menthanylphenyl polyoxyethylene (9.0) ether (TS-90) were synthesized from phenol condensates of a *p*-menthene derived from turpentine oil.

These EO adducts are compounds having 75.0% and 25.0% of the menthanyl and dimenthanyl groups respectively substituted at the *para* and *ortho*, *para* positions of the phenol. The calculated molecular weights (MW) of TS-60, TS-71, TS-80, TS-112, TS-120, TS-131, TS-140, TS-150, TS-165 and TS-200 were 531.24, 579.71, 619.35, 760.33, 795.57, 844.04, 883.69, 927.72, 993.82 and 1147.97 respectively. Calculated MW of TS-90 was 663.42. TS-90, *Anal.* Calcd for C_{36.5}H_{64.5}O₁₀: C, 66.08; H, 9.80. Found: C, 65.77; H, 9.61.

The observed weight-average molecular weight (M_w) and number-average molecular weight (M_n) of TS-90 were 613.3 and 577.6 respectively. The degree of polydispersity ($D = M_w/M_n$) was 1.06.

p-Menthanylphenyl polyoxyethylene (8.8) ether (TS-88) was a 98% monomenthanyl substituted compound prepared by ethoxylating *p*-menthanylphenol, which is obtained by distilling off dimenthanyl substitution products at the *ortho* and *para* positions after phenol condensation reaction. The calculated MW of TS-88 was 620.03. The observed M_w and M_n were 594.7 and 563.2, respectively, D being 1.06.

Sodium dodecyl sulfate (SDS) and nonylphenyl polyoxyethylene (9.0) ether (NNP-90) were obtained from Nikko Chemicals Co., Ltd. of Tokyo, Japan. The hydrophobe of SDS was a linear dodecyl chain and the purity determined from gas chromatography was greater than 99% C₁₂ and the calculated MW was 288.38.

NNP-90 was a compound having 42.9% and 57.1% of the nonyl group respectively substituted at the *ortho* and *para* positions of the phenol. The calculated MW of NNP-90 was 616.84, the observed Mw and Mn were 580.6 and 550.9 respectively and *D* was 1.05.

Isononylphenyl polyoxyethylene (9.0) ether (INP-90) was supplied by Sanyo Kasei Kogyo Co., Ltd. of Kyoto, Japan. The ratio of isononyl group in the *ortho* and *para* positions in INP-90 was 45.1% : 54.9%. The calculated MW was 616.84 and the observed Mw, Mn and *D* were 613.2, 579.4 and 1.06 respectively.

Isooctylphenyl polyoxyethylene (9.0) ether (NOP-90) and isooctylphenyl polyoxyethylene (10.0) ether (NOP-100) were obtained from Nihon Emulsion Co., Ltd. of Tokyo, Japan. The ratio of isooctyl group in *ortho* and *para* positions was 3.9% : 96.1% respectively. The calculated MW of NOP-90 was 602.82 and the observed Mw, Mn and *D* were 591.2, 542.3 and 1.09 respectively. For NOP-100, the calculated MW was 646.87, the observed Mw, Mn and *D* were 661.1, 627.4 and 1.05 respectively.

Diisobutylphenyl polyoxyethylene (10.0) ether (IOP-100) was obtained from Sanyo Kasei Kogyo Co., Ltd. of Kyoto, Japan. The ratio of diisobutyl group in *ortho*, *para* positions was 3.6% : 96.44%. The calculated MW of IOP-100 was 646.87 and the observed Mw, Mn and *D* were 641.2, 608.2 and 1.05 respectively.

Diisobutylphenoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate (BZC) was a product (Hyamine® 1622) of Rohm and Haas Co. of Philadelphia, Pa, U.S.A.

It was supplied by Kimura Sangyo Co., Ltd. of Tokyo, Japan. The calculated MW of BZC was 466.09.

The calculation of the alkyl group distribution in the *ortho* and *para* positions in the non-ionic surfactants described above was made from the peak areas corresponding to the *ortho* and *para* position in the gas chromatograms.* The measurements on average molecular weights Mw and Mn of the ethylene oxide adducts were obtained from the respective peak heights using gel permeation chromatography.** The calculation of average molecular weight and molecular weight distribution was based on a correction curve prepared from 2, 5, 10, 20 and 100 mole standard adducts of nonylphenol.

In addition, to differentiate the alkyl radicals in NNP-90 and INP-90, nuclear magnetic resonance (NMR) was used for the analysis. In the NMR (CDCl₃) spectrum for the samples, approximately same values were obtained in the peak area at 0.3–1.6 ppm due to -CH₃, -CH₂- of the nonyl chain. Thus, it was presumed that the nonyl radical in NNP-90 was probably not completely linear. Since there was no large deviation in the calculated and observed values of molecular weight which would significantly affect the experimental result, the calculated molecular weights were used in this work.

Measurements of Spermicidal Time — The spermicidal test was carried out in accordance with the test for the national certification of contraceptives designated by the Minister.*** The immobilization time of spermatozoa was measured using the procedure described below.

Using the measuring pipet (volume: 1 ml, scale: 0.01 ml), 0.2 ml of the ejaculate obtained from the healthy male donor was transferred to a small test tube (10 mm in diameter and 100 mm long). Next 0.2 ml of the aqueous test sample solution which had been maintained in a constant

* Gas liquid chromatography, Shimadzu GC-RIA, column: Diasolia GT 3 φ, 1 m, detector: FID.

** Hitachi 635 HPLC, column: Shodex GPC A-802 + A-803, dilution solvent: THF, detector: RI.

*** The test method was promulgated on June 30, 1982 by Notification No. 118 of the Ministry of Health and Welfare, Japan.

temperature incubator was added to the ejaculate using the measuring pipet of the same size. The starting point of the spermicidal time measurements of spermatozoa was marked by this addition. After addition the small test tube was shaken until the solution was well mixed. The mixture was dropwise transferred onto three slide glasses (25×75 mm) using the capillary tube, covered with a cover glass (18×18 mm, thickness: 0.17–0.25 mm) and observed under ×200 optical magnification.

A total of 10 optical fields were observed on each sample of the slide glasses and the end point of the spermicidal time was determined when all the spermatozoa became irreversibly immobile.

To determine the spermicidal time (min), tests were repeated three times on each sample and the result was expressed in terms of the average value of the maximum and minimum times required.

The maximum allowable time for observing the spermatozoa under test was set to be 60 min per sample. Concomitantly the sperm which was to be used in the test was inspected for spermicidal time before and after the test using 2.8% (0.14 M) potassium biphthalate in the same manner. This check was intended to confirm the normality of the sperm's biologic activity. When the spermicidal time of spermatozoa was found in either inspection to be less than five minutes, the test results with the group in which the spermatozoa were included were not used in evaluation.

In the comparative study of the nine compounds, a serial dilution of each surfactant was carried out with 5% (w/v) glucose solution (glucose injection, JPX), and the tests were conducted at ambient temperature (15–25°C).^{*} In the experiments, the individual difference in human spermatozoa was taken into consideration. To

obtain objective data on spermicidal activity of the test samples, human ejaculate was obtained from two healthy male donors. To determine the spermicidal time, tests were repeated three times on each sample from each donor.

The comparative study of various EO adducts of *p*-menthanylphenol, on the other hand, was conducted at 37°C. Before using each surfactant was dissolved in freshly distilled water (distilled water for injection, JPX).

In the evaluation of the spermicidal activity of the surfactants under study, the mean values of spermicidal time obtained from the above experiments was used.

Surface Tension Measurements — The surface tension for each surfactant was determined with a standard capillary tube pipet specified by Japanese Industrial Standards** using drop weight method. The drop weight of each liquid sample was measured in 5% (w/v) glucose solution (glucose injection, JPX).

The drop weight was converted into surface tension by determining the standard surface tension (JIS) of distilled water with the same capillary tube pipet. Three measurements were taken from each sample and the average value was used. In addition, the specific gravity of each sample was determined for the correction of the drop weight. The measurements were taken at 25±0.1°C by immersing the apparatus in a constant temperature water bath. The critical micelle concentration (cmc) of each surfactant was obtained from the plot of surface tension *versus* surfactant concentration.

Wetting Rate Measurements — The rate of wetting was determined by measuring the sinking time of woolen felt sample specified by Japanese Industrial Standards*** as described previously.⁷⁾ In the comparative study of various EO adducts of *p*-menthanylphenol, each surfactant was dissolved in freshly distilled water (distilled

* Ordinary temperature specified by JPX.

** JIS K 3362-1970, volume: 5 ml, capillary tube length: 60 mm, inner diameter: 0.5 mm, outer diameter: 6.0 mm.

*** JIS L 3201-1966, white color, No. 1. The woolen felt was supplied by Nippon Keori Kaisha Ltd. of Kobe, Japan, in the standard of No. 1100.

water for injection, JPX) and the determination was carried out at $37 \pm 0.5^\circ\text{C}$. The average weight ($n=20$) of the felt test pieces ($20 \times 20 \times 3$ mm) used in the test was 366.6 ± 15.6 mg (S.D.) and the 95% confidence interval was $359.3-373.9$ mg.

The comparative study of the nine compounds, on the other hand, was carried out at $25 \pm 0.5^\circ\text{C}$. Each surfactant was dissolved in 5% (w/v) glucose solution (glucose injection, JPX). Furthermore, 391.2 ± 5.4 mg (S.D.) and the 95% confidence interval was $388.7-393.7$ mg.

*Partitioning of Nonionic Surfactants between Water and *n*-Octanol* — For each of seven nonionic surfactants tested, 0.2 g of each sample was dissolved in 50 ml of distilled water, and placed in a glass-stoppered Erlenmeyer flask with the addition of 50 ml of *n*-octanol. The flask, immersed in a constant temperature water bath at $25 \pm 0.5^\circ\text{C}$ was mechanically shaken for two hours. Immediately after, each mixture was centrifuged at 3000 rpm for three minutes.

Samples from the upper organic phase and lower aqueous phase were taken with a pipet. The organic phase and the aqueous samples were respectively diluted with *n*-octanol and anhydrous ethanol. By determining the concentrations of the samples with a UV spectrophotometric method ($A:A_{275}$), the concentration distribution in the organic and aqueous phases was obtained. Three measurements were made on each sample and the partition ratios were expressed in terms of the mean value and standard deviation (S.D.). In addition, *n*-hexane was also used in place of *n*-octanol for similar tests in four of the seven surfactants evaluated.

Calculation of Minimum Spermicidal Concentration — Since it was observed that there was a nonlinear relationship between spermicidal time (t) and surfactant concentration (c), exponential functions, $c = Ae^{-\alpha t}$ and $c = Ae^{-\alpha t} + Be^{-\beta t}$ were chosen as the equations for this study. A digital computer was employed to plot regression curves, the minimum spermicidal effective concentrations of the surfactant at five and zero min were calculated.

Olivetti 6060 computer was used for the initial exponential estimates for the purpose of statistical analysis and IBM 3032-008 computer was used for calculating other parameters. To evaluate the extent of fit of the estimates and nonlinear regression curves, computer program of Sedman *et al.*⁸⁾ was used to calculate F -values and R -squared values (r^2).

RESULTS AND DISCUSSION

The spermicidal effectiveness of the ionic and nonionic surfactants at various concentrations and the surface tensions measured in this work are presented in Table I and II.

Spermicidal Effect

To objectively determine the effective concentrations for the surface-active spermicides, the data corresponding to zero minute and greater than 60 min were omitted from the experimental data given in Table I and II. Using the least squares method, the regression curves were plotted from the data with a digital computer. The minimum spermicidal concentrations for irreversibly immobilizing all spermatozoa in 5 min (MSC_5) and 0 min (MSC_0), calculated from the method described in Materials and Methods are given in Table III.

From the F -values and R -squared values calculated for each sample, the statistical analysis generally gave a good fit for the experimental data except for SDS, NOP-100 and TS-88. When the spermicidal potencies of the test samples were compared with objectively determined MSC_0 , the relative order can be given as follows: $\text{INP-90} > \text{NNP-90} > \text{TS-88}, \text{TS-90} > \text{NOP-90} > \text{IOP-100} > \text{NOP-100}, \text{BZC} > \text{SDS}$.

Some nonionic surfactants used in this investigation are the same ones regarded safe and effective (Category I) by the U.S. Food and Drug Administration's OTC Review on vaginal contraceptive drug products. They are known by their generic names as menfegol (*p*-methanylphenyl polyoxyethylene (8.8) ether), nonoxynol-9 (nonylphenoxy polyethoxy ethanol) and octoxynol-9 (*p*-diisobutyl phenoxy polyethoxy ethanol).

According to this FDA report,⁹⁾ the effective

concentrations for menfegol (TS-88), nonoxynol-9* and octoxynol-9** for totally immobilizing all spermatozoa within 20 s were 0.38 mg/ml, 0.12 mg/ml and 0.24 mg/ml respectively. Compared to our results, these concentrations are generally lower and octoxynol-9 was given much higher spermicidal effectiveness.

One reason for the discrepancy is probably due to the difference in chemical compositions in the commercially available surfactants. For example, both INP-90 and NNP-90 are equivalent to nonoxynol-9 but they exhibited some difference in their spermicidal efficacy. Additionally, slight differences in the experimental procedure used for sperm immobilization according to the method of Sander and Cramer¹⁰⁾ might have also caused the discrepancy.

In the above experimental results, whereas

the effective concentration for 5 min spermicidal time for the cationic surfactant BZC was relatively low (1.60 mM) and comparable to that of nonionics with 9 mol ethylene oxide, the effective concentration of BZC for 0 min was approximately twice (2.94 mM).

From our past experimental results and other investigations, it was found that the spermicidal concentration³⁾ against human spermatozoa for the cationic surfactant was higher than the erythrocytes hemolytic concentration¹¹⁾ or the bactericidal concentration^{3,12)} for certain microbes. Thus, the biological activity of the cationic surfactant against spermatozoa can be regarded as relatively weak. It was postulated that the reason was due to the fact that the composition and surface charge of the surface cell membrane of spermatozoa¹³⁾ differed from

TABLE I. *Spermicidal Time and Surface Tensions in Various Concentrations of Ionic Surfactants*

Surfactants	Concentration ^{a)} (mM)	Spermicidal time ^{b)} (min)	Surface tension (dyn/cm)
SDS	10.000	0	38.49
	5.460	2–3	43.80
	5.000	20–23	45.53
	3.333	25–30	53.00
	2.500	60	57.73
	1.250	>60	66.01
BZC	5.000	0	32.27
	3.333	0	37.67
	2.500	2–3	39.75
	1.667	4–5	44.00
	1.250	8–10	48.30
	1.000	15–20	52.14
	0.833	45–50	54.33
	0.714	60	56.33
	0.625	>60	57.60
Control ^{c)}	0	>60 ^{d)}	72.38

a) Concentration in 5% glucose solution at 25°C.

b) Values represent the minimum and maximum range of the mean values obtained from three replicates from two donors; zero minute indicates 30 s or less.

c) 5% glucose solution. d) $p=0.05$.

* Tergitol® TP-9, Union Carbide Corp., New York, NY, USA.

** Triton® X-100, Rohm & Haas Co., Philadelphia, Pa, USA.

TABLE II. *Spermicidal Time and Surface Tensions in Various Concentrations of Nonionic Surfactants*

Surfactants	Concentration ^{a)} (mM)	Spermicidal time ^{b)} (min)	Surface tension (dyn/cm)
TS-88	5.000	0	34.15
	2.500	0	34.39
	1.667	0	34.67
	1.250	2–3	35.33
	1.000	35–40	35.92
	0.833	50–55	36.67
	0.714	50–58	37.50
	0.625	60	38.30
	0.500	>60	39.90
TS-90	5.000	0	34.15
	2.500	0	35.01
	1.667	0	35.67
	1.250	2–3	36.17
	1.000	20–25	36.74
	0.833	50–55	37.67
	0.714	50–58	39.00
	0.625	60	40.00
NNP-90	5.000	0	30.31
	2.500	0	30.29
	1.667	0	31.00
	1.250	0	31.16
	1.000	2–3	31.19
	0.714	30–35	33.00
	0.625	45–48	33.66
	0.500	60	34.89
INP-90	5.000	0	31.29
	2.500	0	31.39
	1.667	0	31.66
	1.250	0	31.67
	1.000	0	31.91
	0.833	1–2	32.00
	0.714	25–28	32.50
	0.625	35–40	32.33
	0.500	60	34.64
NOP-90	5.000	0	30.89
	2.500	0	31.09
	1.670	2–4	31.15
	1.250	30–35	31.20
	1.000	60	31.29
	0.830	>60	33.66

NOP-100	5.000	0	33.33
	2.500	2-3	33.44
	1.250	23-25	33.83
	0.625	60	35.00
	0.313	>60	39.30
IOP-100	5.000	0	32.77
	2.500	0	32.77
	2.273	1-2	32.77
	2.000	5-6	32.80
	1.667	14-17	32.83
	1.250	27-30	32.83
	0.625	60	34.67
	0.313	>60	39.00
Control ^{c)}	0	>60 ^{d)}	72.38

a) Concentration in 5% glucose solution at 25°C.

b) Values represent the minimum and maximum range of the mean values obtained from three replicates using samples from two donors; zero minute indicates 30 s or less.

c) 5% glucose solution. d) $p=0.05$.

TABLE III. *Spermicidal Effects of Surfactants in 5% Glucose Solution at 25°C*

Surfactants	$n^a)$	$MSC_5^b)$ (mM)	$MSC_0^c)$ (mM)	Statistical analysis	
				F -value ^{d)}	$RS^e)$
SDS	4	5.40	5.79 ± 0.67	745.25	0.846
BZC	5	1.60	2.94 ± 1.04	3.67	0.997
TS-88	5	1.25	1.31 ± 0.09	96.76	0.898
TS-90	5	1.21	1.27 ± 0.05	34.88	0.964
NNP-90	4	0.97	1.03 ± 0.02	2.82	0.995
INP-90	4	0.82	0.86 ± 0.02	4.86	0.979
NOP-90	3	1.63	1.71 ± 0.02	2.02	0.998
NOP-100	3	2.30	2.64 ± 0.19	118.61	0.984
IOP-100	5	2.07	2.31 ± 0.03	17.43	0.998

a) The total number of data points.

b, c) Estimated minimum spermicidal effective concentration at spermicidal time in 5 min and 0 min. MSC_0 values represent mean $\pm$ S.D.

d) Sum of the squared deviation.

e) R -squared (r^2) were calculated by the equation of Sedman *et al.*⁸⁾

that of erythrocytes¹⁴⁾ or the microbes.¹⁵⁾ In addition, the medium of the semen might also have an inhibitory action on the biological activity of the surfactant.

Recently, Chvapil *et al.*¹⁶⁾ reported an interesting work on the biological toxicity of nonoxynol-9* on human spermatozoa and human fibroblasts (embryonal lung WI-38) and

* Igepal® CO-630, GAF Corp., New York, NY, USA.

TABLE IV. Correlation between Spermicidal Time (*Y*) and Surface Tensions (*X*) of Surfactants in 5% Glucose Solution at 25°C

Surfactants	<i>r</i> ^{a)}	<i>n</i> ^{b)}	Statistical analysis ^{c)}	Regression equation
SDS	0.934	5	<i>p</i> <0.05	<i>Y</i> =2.95 <i>X</i> −118.65
BZC	0.863	8	<i>p</i> <0.01	<i>Y</i> =2.51 <i>X</i> −97.93
TS-88	0.944	8	<i>p</i> <0.001	<i>Y</i> =17.23 <i>X</i> −592.12
TS-90	0.930	8	<i>p</i> <0.001	<i>Y</i> =12.74 <i>X</i> −447.77
NNP-90	0.963	7	<i>p</i> <0.01	<i>Y</i> =14.35 <i>X</i> −440.50
INP-90	0.972	9	<i>p</i> <0.001	<i>Y</i> =19.89 <i>X</i> −627.77
NOP-90	0.783	5	NS	<i>Y</i> =139.10 <i>X</i> −4310.29
NOP-100	0.993	4	<i>p</i> <0.01	<i>Y</i> =36.07 <i>X</i> −1201.09
IOP-100	0.897	7	<i>p</i> <0.01	<i>Y</i> =27.93 <i>X</i> −907.72

a) Correlation coefficient.

b) The total number of data points.

c) Statistical evaluations were performed by the regression analysis of variance; NS means not significant (*p*> 0.05).

TABLE V. Critical Micelle Concentration and Surface Tensions of Surfactants in 5% Glucose Solution at 25°C

Surfactants	cmc ^{a)} (mM)	γ_{cmc} ^{b)} (dyn/cm)	γ_{scc} ^{c)} (dyn/cm)
SDS	7.4 (5.0–10.0)	40.0	40.2
BZC	3.5 (2.0–5.0)	35.0	39.0
TS-88	0.7 (0.5–1.0)	36.0	34.4
TS-90	0.7 (0.5–1.0)	37.0	34.9
NNP-90	0.4 (0.3–1.0)	32.0	30.7
INP-90	0.6 (0.5–1.0)	32.0	31.6
NOP-90	0.4 (0.3–1.0)	31.5	31.0
NOP-100	0.5 (0.3–1.0)	34.0	33.3
IOP-100	0.5 (0.3–1.0)	33.0	32.5

a) Critical micelle concentration. b) Surface tensions at cmc.

c) Surface tension at the concentration that immobilizes of sperm in zero time.

compared it against other representative anionic surfactants, SDS* and cationic surfactant, cetylpyridinium chloride**. According to their experiments, the relative order of cytotoxicities against spermatozoa and fibroblasts was: cationic > nonionic > anionic.

The cationic surfactant showed a higher biological effect than nonionic, nonoxynol-9 in their work. The reason for the discrepancy in their results and ours could have been due to the methodological differences. In their work, a special buffer solution containing electrolytes was

* Sodium dodecyl sulfate, N-5750, Sigma Chemical Co., St. Louis, Mo, USA.

** Cepacol®, Sigma Chemical Co., St Louis, Mo., USA.

used to dilute the human ejaculates to 1 : 100, and the observation was made until more than 95% of the spermatozoa were immobilized.

Spermicidal Activity and Surface Tension

A portion of the data, showing spermicidal time greater than 60 min in Table I and II, was discarded and the spermicidal time (Y) was plotted against surface tension (X). The result, shown in Table IV, indicated a significant linear correlation of the variables except for NOP-90.

As can be seen from the regression equation shown in Table IV, for NOP-90, NOP-100 and 10P-100, the slopes of the straightline of Y versus X relation are relatively steep with these three compounds. This fact suggests that their spermicidal time is greatly influenced by their surface tension. Nevertheless, as is obvious from the data shown in Table III, these three compounds were less potent than the other nonionics tested.

In addition, the values of X at $Y = 0$ that were derived from the regression equations shown in Table IV, that is, the estimated values of surface tension at the concentration that immobilizes of sperm in zero time (γ_{sc}) are shown

in Table V together with the values cmc and surface tension at cmc (γ_{cmc}).

From the results of Table V, it can be observed that ionic surfactants such as anionic SDS and cationic BZC showed spermicidal activities at concentrations slightly below the cmc's while the nonionic surfactants exhibited activities at concentrations above critical concentrations for micellization. Moreover, among nonionic surfactants, while the nonylphenol and *p*-menthanylphenol derivatives showed sufficient spermicidal effectiveness at concentrations equivalent to approximately twice their cmc's, the octylphenol derivatives required approximately four times their respective cmc's.

When the nine compounds including ionic compounds were studied, there was a significant correlation between γ_{sc} and molar MSC_0 in Table III, ($r=0.789$, $n=9$, $p<0.05$) and for these compounds there was also a significant correlation between cmc and molar MSC_0 ($r=0.915$, $n=9$, $p<0.05$). However, when the series of seven nonionic compounds was considered, no such significant relationship was noted. Nor

TABLE VI. *Spermicidal Time and Wetting Time of Various Ethylene Oxide Adducts of p-Menthanylphenol in 5.0 mM Conc. Aqueous Solution at 37°C*

Surfactants	mol of EO	Wetting time (X) (s)	Spermicidal time (Y) (min)
TS-60	6.0	10.3	3-4
TS-71	7.1	8.5	2-3
TS-80	8.0	6.1	0-1
TS-90	9.0	4.1	0
TS-112	11.2	4.8	0
TS-120	12.0	4.5	0-1
TS-131	13.1	6.2	3-4
TS-140	14.0	9.0	4-5
TS-150	15.0	12.8	5-6
TS-165	16.5	15.0	5-7
TS-200	20.0	31.0	18-20
Control ^{a)}		>600	>60 ^{b)}

Values represent the minimum and maximum range of the mean values obtained from three replicates; zero minute indicates 30 s or less.

a) Distilled water. b) $p=0.05$.

was there any significant relationship between γ_{cmc} and molar MSC_0 when all the nine compounds were compared ($p > 0.05$).

It can therefore be said that the surface tension and cmc, determined from surface tension as a function of molar concentrations, are not relevant as direct measures of the spermicidal action of the nonionic surfactants studied.

From earlier studies by Kondo and Tomizawa¹⁷⁾ on hemolytic action of surfactants, which indicated that the hemolytic concentrations were independent of the surface tensions of the solutions, the possibility of a surface pressure mechanism in effect can be ruled out. Moreover, considering the fact that there was no apparent direct correlation between surface activity and hemolytic activity, it would be difficult to demonstrate generally valid relationship between cmc and spermicidal activity among the series of nonionic surfactants from the results of Table V which provided data at one temperature (25°C) using nonionic surfactants having similar physical properties.

Spermicidal Efficacy and Wetting Efficiency

The spermicidal time and rate of wetting by 6.0- through 20.0-mol EO adducts of *p*-menthanylphenol in the 5.0 mM aqueous solu-

tion are shown in Table VI.

There was a significant linear correlation between wetting time (t_s) and spermicidal time (t_{sc}) of these EO adducts, which can be represented by equation 1 ($r = 0.983$, $n = 11$, $p < 0.001$).

$$t_{sc} = 0.658 t_s - 2.85 \quad (\text{Eq. 1})$$

It was then found that 9.0- through 11.2-mol EO adducts took the shortest spermicidal time and that 9.0- mol EO adducts had the shortest wetting time as shown in Table VI. Previously, the authors reported that the EO 7.0-mol adduct had the lowest surface tension of all EO adducts studied.⁷⁾ When this fact is coupled with the results of the present study, it is suggested that the spermicidal effect of the EO adduct of *p*-menthanylphenol against human spermatozoa is associated more closely with its wetting time than with its surface tension.

According to Fowkes,¹⁸⁾ the interface tension and contact angle of surfactants can be indirectly known by observing their wetting efficiency. To determine whether his theory would apply to the compounds under study, an experiment was undertaken with a representative surfactant, TS-90, which is known to have a linear relation-

TABLE VII. Wetting Time of Surfactants in 5% Glucose Solution at 25°C

Surfactants	Wetting time (s)	
	5.0 mM conc.	1.0 mM conc.
SDS	12.05 ± 0.67	>600.00
BZC	3.85 ± 0.10	32.64 ± 8.52
TS-88	8.47 ± 0.26	19.47 ± 1.09
TS-90	8.70 ± 0.12	19.97 ± 1.78
NNP-90	5.55 ± 0.02	13.21 ± 0.96
INP-90	5.44 ± 0.14	13.02 ± 0.80
NOP-90	3.97 ± 0.15	10.39 ± 0.26
NOP-100	3.94 ± 0.30	14.88 ± 1.22
IOP-100	3.97 ± 0.52	12.04 ± 1.16
Control ^{a)}	>600.00	>600.00

Each value represents mean ± S.D. of three replicates.

a) 5% glucose solution.

ship between logarithmic molar concentration and logarithmic wetting time.

The experimental results of wetting time for felt test pieces using different surfactants at 5.0 mM and 1.0 mM concentrations are given in Table VII.

The wetting time data at 5.0 mM were compared with the molar MSC_0 in Table II to test the relationship but not statistically significant correlation was found for the samples tested ($p > 0.05$). However, the wetting time for the lower concentration (1.0 mM) suggested a possible relationship with spermicidal potency. Particularly, for the series of nonionic surfactants with the same hydrophobe, the spermicidal potency was clearly related to the wetting efficiency.

Relation between Spermicidal Effect and Partition Ratio

The partition ratios of the nonionic surfactants between *n*-octanol and water are shown in Table VIII. The partition ratios reported here were apparent partition ratios (APR) obtained by dividing the partition concentrations in *n*-octanol by partition concentrations in the aqueous phase and the interface.

The APR values appear to follow the relative effective order of MSC_0 values of nonionic surfactants shown in Table III. However, no statistically significant relationship was found between

APR values and logarithms ($\log c$) of molar MSC_0 given in Table III ($p > 0.05$). The reason is probably due to the fact that the samples were hydrophilic and had good solubility in polar solvent, *n*-octanol, and thus APR values did not show much variation.

The partitioning of four compounds having different hydrophobic groups between *n*-hexane and water are also shown in Table VIII.

The partition coefficient (PC) was calculated by dividing the surfactant distribution concentration in *n*-hexane phase by the concentration in the aqueous phase, neglecting the small amount of the surfactant absorbed at the interface.

A significant linear correlation was obtained between PC and the logarithms ($\log c$) of molar MSC_0 given in Table III, for $PC > 0$, which approximately obeyed equation 2 ($r = -0.957$, $n = 4$, $p < 0.05$).

$$\log c = -2.22PC - 2.12 \quad (\text{Eq. 2})$$

The above result represents an advancement of knowledge over the study of Harvey and Stuckey^{2c)} who failed to formulate a definite relationship between spermicidal effect and the solubility data of surfactants in *n*-hexane obtained by preparing a saturated solution.

In conclusion, we obtained several findings of interest in the present study. In various nonionic surfactants tested, the difference in the chemical

TABLE VIII. *Partition Ratios of Nonionic Surfactants between Organic Solvents and Water*

Surfactants	<i>n</i> -Octanol/water system APR ^{a)}	<i>n</i> -Hexane/water system PC ^{b)}
TS-88	7.13 ± 2.65	0.313 ± 0.023
TS-90	5.16 ± 0.13	
NNP-90	6.87 ± 0.54	
INP-90	6.48 ± 0.10	0.436 ± 0.001
NOP-90	6.58 ± 0.65	0.282 ± 0.009
NOP-100	4.48 ± 0.11	
NOP-100	4.63 ± 0.11	0.260 ± 0.008

a) Apparent partition ratio, values represent mean ± S.D.

b) Partition coefficient of the nonionic surfactant having different hydrophobic groups. Values represent mean ± S.D.

structure of the hydrophobes in the configuration of the alkyl chain could cause a difference in spermicidal activity. The importance of the structural effect was particularly evident in octylphenyl polyoxyethylene series and nonylphenyl polyoxyethylene series. The result indicates the importance for clarifying the chemical composition when using commercially available nonionic surfactants for evaluating biological activity.

There was a significant correlation between surface tension and wetting time on the one hand and spermicidal potency, when the surfactants were considered individually. For all the compounds as a whole, however, the physical properties of these compounds cannot be considered relevant parameters of the spermicidal potency of surface-active spermicides.

On the other hand, it has been demonstrated in this study that the partition ratio between *n*-hexane and water can be a direct measure of the spermicidal action of nonionic surface-active spermicides.

Our experience indicates that any valid measure of the biological activity, especially structure-related activity, of surfactants against spermatozoa cannot be derived from their inherent physical properties alone.

It has been revealed by many investigators that the major lipids contained in sperms of various species of animals are phospholipids.^{13a)} It follows that the effect of the surfactant on the motility of sperms in their primary stage of activity is derived from its action on the phospholipids, the main constituent of the sperm cell surface membrane. This assumption readily leads to the postulation that, as is the case with erythrocytes^{11,17,19)} and microbes,^{12d)} the potency of surfactants, especially nonionic ones, used at low concentrations is dependent on their interaction mainly with the lipids in the sperm cell surface membrane and that their interaction varies with the chemical structure of their hydrophobes.

It can be inferred from the results of the experiment concerning the partition ratio between

n-hexane and water that the spermicidal activity of nonionic surfactants is related to their affinity to lipids in the sperm cell surface. In other words, the results of the present study suggest that the spermicidal activity of surfactants is related to their amphipathic molecular structure, as is the case with their antimicrobial activity that has been reported by other investigators to be also associated with their chemical structure.²⁰⁾

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REFERENCES

- 1) a) J.R.Baker: The spermicidal powers of chemical contraceptives, *J. Hyg.*, **29**, 323–329 (1930); b) V.R.Swayne, J.M.Beiler and G.J.Martin: Spermicidal effect of certain physiologically active substances, *Proc. Soc. Exp. Biol. Med.*, **80**, 384–386 (1952); c) H.H.Koeffoed-Johansen and T.Mann: Studies on the metabolism of semen, *Biochem. J.*, **57**, 406–410 (1954); d) P.E.Lindahl and K.Wedin: Registration of the spermicidal effects of dioctyl sodium sulphosuccinate, *Nature* (London), **184**, 1289–1291 (1959).
- 2) a) J.R.Baker, R.M.Ranson and J.Tynen: The spermicidal powers of chemical contraceptives, *J.Hyg.*, **37**, 474–488 (1937); b) V.R.Swayne, J.M.Beiler and G.J.Martin: Spermicidal effect of certain physiologically active substances, *Proc. Soc. Exp. Biol. Med.*, **80**, 384–386 (1952); c) C.Harvey and R.E.Stuckey: Spermicidal activity of surface-active agents, *J. Reprod. Fertil.*, **3**, 124–131 (1962); d) D.A.Berberian, W.G.Gorman, H.P.Drobeck, F.Coulston and R.G.Slighter, Jr.: The toxicology and biological Properties of Laureth 9 (a polyoxyethylene lauryl ether), a new spermicidal agent, *Toxicol. Appl. Pharmacol.*, **7**, 206–214 (1965).
- 3) S.Iwahara, C.Ishizeki and K.Furuse: Studies on the surface active agents as spermicides, *Jpn. J. Public Health*, **12**, 123–129 (1965).

- 4) S.Iwahara, C.Ishizeki and K.Furuse: Antagonistic effect of spermicidal surfactants, *Med. Biol.*, (Tokyo), **73**, 98–101 (1966).
- 5) S.Iwahara, C.Ishizeki and K.Furuse: Physical and biological properties of nonionic surfactants, *Eisei Shikensho Hokoku*, **85**, 81–83 (1967).
- 6) M.Kawane, E.Fujiwara and K.Furukawa: Japan Patent 3926198 (1964).
- 7) K.Furuse: Étude sur nouveau surfactif non ionique, Un dérivé du *p*-menthanylphényl polyoxyéthylène, *Parf. Cosm. Sav.*, **10**, 342–347 (1967).
- 8) A.J.Sedman and J.G.Wagner: CSTRIP, a Fortran IV computer program for obtaining initial polyexponential parameter estimates, *J. Pharm. Sci.*, **65**, 1006–1010 (1976).
- 9) Food and Drug Administration (ed.): Varinal contraceptive drug products for over-the-counter human use; Establishment of a monograph; Proposed rulemaking, *Fed. Regist.*, **45**, 82014–82049 (1980); R.E.Homm, G.E.Doscher, E.J.Hummel and F.C.Green-slade: Relative antifertility activity of three vaginal contraceptive products in the rabbit: Relationship to *in vitro* data, *Contraception*, **13**, 479–488 (1976).
- 10) F.V.Sander and S.D.Cramer: A practical method for testing the spermicidal action of chemical contraceptives, *Hum. Fertil.*, **6**, 134–138 (1941).
- 11) T.Kondo and M.Tomizawa: Mechanism of hemolysis by cationic surface-active agents, *J. Pharm. Sci.*, **58**, 1378–1381 (1969).
- 12) a) G.Domagk: Eine neue Klasse von Desinfektionsmitteln, *Dtsch. Med. Wochenschr.*, **61**, 829–832 (1935); b) Z.Baker, R.W.Harrison and B.F.Miller: Action of synthetic detergents on the metabolism of bacteria, *J. Exp. Med.*, **73**, 249–271 (1941); c) *Idem*: The bactericidal action of synthetic detergents, *ibid.*, **74**, 611–620 (1942); d) C.A.Lawrence, C.E.Kwartler, V.L.Wilson and E.W.Kivela: Germicidal action of some benzyl quaternary ammonium compounds having substituents in the aromatic nucleus, *J. Am. Pharm. Assoc.*, **35**, 357–358 (1947); e) W.B.Hugo: The mode of action of antiseptics, *J. Pharm. Pharmacol.*, **9**, 145–161 (1957).
- 13) a) F.Miescher: Physiologisch-chemische Untersuchungen über die Lachsmilch, *Schmiedeberg's Archiv*, **37**, 100–155 (1896); E.F.Hartree and T.Mann: Phospholipids in ram semen: Metabolism of plasmalogen and fatty acid, *Biochem. J.*, **80**, 464–476 (1961); T.W.Scott, R.M.C.Dawson and I.W.Rowlands: Phospholipid interrelationships in rat epididymal tissue and spermatozoa, *Biochem. J.*, **87**, 507–512 (1963); T.W.Scott, J.K.Voglmayr and B.P.Setchell: Lipid composition and metabolism in testicular and ejaculated ram spermatozoa, *Biochem. J.*, **102**, 456–461 (1967); b) I.Ishizuka, M.Suzuki and T.Yamakawa: Isolation and characterization of a novel sulfoglycolipid, 'Seminolipid', from boar testis and spermatozoa, *J. Biochem.*, **73**, 77–87 (1973); K.Ueno, I.Ishizuka and T.Yamakawa: Glycolipid composition of human testis at different ages and the stereochemical configuration of seminolipid, *Biochim. Biophys. Acta*, **487**, 61–73 (1977).
- 14) D.G.Cornwell, R.E.Heikkila, R.S.Bar and G.L.Blagi: Red blood cell lipid and the plasma membrane, *J. Am. Oil. Chem. Soc.*, **45**, 297–304 (1968); P.Zahler: The structure of the erythrocyte membrane, *Experientia*, **25**, 449–560 (1969); M.R.Bretscher: A major protein which spans the human erythrocyte membrane, *J. Mol. Biol.*, **59**, 351–357 (1971); M.J.A.Tanner and D.H.Boxer: Separation and some properties of the major proteins of the human erythrocytes membrane, *Biochem. J.*, **129**, 333–347 (1972); J.P.Segrest, I.Kahane, R.L.Jackson and V.T.Marchesi: Major glycoprotein of the human erythrocyte membrane; Evidence for an amphipathic molecular structure, *Arch. Biochem. Biophys.*, **155**, 167–183 (1973).
- 15) G.J.Gittens and A.M.James: Some physical investigations of the behaviour of bacterial surfaces VI. Chemical modification of surface components, *Biochim. Biophys. Acta*, **66**, 237–249 (1963); R.Sato: Biological chemistry. V. Structure and function of biological membranes 1., *Kagaku to Kogyo* (Tokyo), **23**, 1118–1124 (1970); A.Tomasz: The bacterial cell surface, *Nature* (London), **234**, 389–392 (1971); L.Rothfield and D.Romes: Role of lipids in the biosynthesis of the bacterial cell envelope, *Bacteriol. Rev.*, **35**, 14–38 (1971).
- 16) M.Chvapil, J.B.Ulreich, K.O'dea, K.Betts and W.Droegemueeller: Studies on nonoxynol-9. III. Effect on fibroblasts and spermatozoa, *Fert. Steril.*, **33**, 521–525 (1980).
- 17) T.Kondo and M.Tomizawa: Hemolysis by nonionic surface-active agents, *J. Pharm. Sci.*, **57**, 1246–1248 (1968).
- 18) F.M.Fowkes: Role of surface active agents in wetting, *J. Physical Chem.*, **57**, 98–103 (1953).
- 19) M.Tomizawa and T.Kondo: Mechanism of hemolysis by anionic surface-active agents, *Kolloid-Z.Z. Polym.*, **246**, 694–699 (1971); B.Y.Zaslavsky, N.N.Ossipov, V.S.Krivich, L.P.Baholdina and S.V.Rogozhin: Action of surface-active substances on biological membranes II. Hemolytic activity of nonionic surfactants, *Biochim. Biophys. Acta*, **507**, 1–7 (1978).
- 20) C.Hansch and E.J.Lien: Structure-Activity relationships in antifungal agents. A survey, *J. Med. Chem.*, **14**, 653–670 (1971).